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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAY 2 1995

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 625 6(A)(2). Thiophanate-methyl. Review of Chronic/Oncogenicity Feeding Study in Rats

PC Code 102001
Tox. Chem. No. 375 A
Project No. D195040
Submission No. S448036
MRID No. 428966-01

TO: Shanaz Bacchus
Accelerated Reregistration Branch
Special Review and
Reregistration Division (7508W)

FROM: Pamela M. Hurley, Toxicologist Pamela M. Hurley 4/17/95
Section I, Toxicology Branch I
Health Effects Division (7509C)

THRU: Roger L. Gardner, Section Head Roger Gardner 4-27-95. KB 4/27/95
Section I, Toxicology Branch I
Health Effects Division (7509C)

Background and Request:

Elf ATOCHEM North America has submitted a combined chronic feeding/oncogenicity study for thiophanate-methyl in response to reregistration requirements. The study has been classified as 6(A)(2) data. The Toxicology Branch (TB-I) has been asked to review and comment on the study.

Toxicology Branch Response:

The Toxicology Branch has reviewed the combined chronic feeding/oncogenicity study for thiophanate-methyl and has determined that it satisfies the regulatory requirement for toxicology testing guideline number 83-5 for thiophanate-methyl (chronic feeding/oncogenicity study in rats). The study is classified as Core Guideline. TB-I notes, however, that the way the study was reported made interpretation more difficult and time consuming. It would have been helpful if tables with combined incidences from both the terminal sacrifice and the unscheduled deaths for each of the macroscopic and microscopic

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data tables were included in the report. In addition, in order to complete our analysis, TB-I is requesting two items: historical control data for adrenal pheochromocytomas in males and mononuclear cell leukemia in the spleen of females and the reports on the mechanistic investigation of the effect of thiophanate-methyl on the thyroid and liver which were summarized in Annex 5 of the combined chronic toxicity/oncogenicity study in rats. The following paragraphs summarize the results of the study.

In a 2-year feeding/oncogenicity study, thiophanate methyl was administered in the diet to 60 male and 60 female F344 rats/group at 0, 75, 200, 1200, or 6000 ppm. After week 52, 10 rats/sex/dose were sacrificed, except only five 6000 ppm males were sacrificed because 8 males died from non-treatment related injury at weeks 11 and 12. The mean compound consumption for the study was 0, 3.3, 8.8, 54.4, and 280.6 mg/kg/day for males and 0, 3.8, 10.2, 63.5, and 334.7 mg/kg/day for females.

Rats fed 75 and 200 ppm had no significant treatment-related toxic effects. Male rats fed 1200 ppm and 6000 ppm had significantly decreased mean body weights and net weight gains at the end of the study. The mean weight of 1200 ppm males was 84% of controls ($p<0.001$), and the net gain was 79% of controls ($p<0.001$), whereas the two 6000 ppm males which survived to week 104 had a mean weight 73% of controls and net weight gain 63% of controls. Female rats had significant body weight changes only in the 6000 ppm dose group, the mean weight was 78% ($p<0.001$) and the mean net gain was 69% ($p<0.001$) of controls at the end of the study. The 1200 and 6000 ppm males and females had decreased food efficiency. Food efficiency in rats fed 1200 ppm was reduced to 78% and 88% of controls in males and females, respectively, while in 6000 ppm rats, the efficiencies were lowered to 65% and 71% in males and females. There was a treatment-related decrease in survival in only the 6000 ppm group males (2/55 survivors vs. 37/50 controls, $p<0.001$); the marginal increase in mortality ($p<0.05$) in the 200 ppm group males appeared spurious. Other male groups and all female dose groups were unaffected. Non-neoplastic pathological changes were observed primarily in 1200 and 6000 ppm rats in the liver (dose-related weight increase and hepatocellular hypertrophy), kidney (surface changes, dose-related increase in weight and severity of nephropathy), and thyroid (dose-related weight increase, follicular cell hypertrophy and hyperplasia, and T_3 and T_4 hormone level decreases). The levels of thyroid stimulating hormone (TSH) were elevated, though pituitary weights were unchanged. **A LOEL of 1200 ppm was identified for both male (54.4 mg/kg/day) and female (63.5 mg/kg/day) rats, based on treatment-related effects in the liver, kidneys, and thyroid. The corresponding NOEL was 200 ppm in both sexes of rats (corresponding to 8.8 mg/kg/day for males and 10.2 mg/kg/day for**

females), based on lack of significant toxic effects at this dose.

The toxic effects observed in the thyroid in 1200 and 6000 ppm male and female rats were accompanied by a dose-related increase in the incidence of follicular cell adenoma (males: 1/50, 0/48, 0/50, 3/50, 12/55 and females: 0/50, 0/49, 0/50, 1/50, 2/50 for doses of 0, 75, 200, 1200, and 6000 ppm, respectively). The increase was statistically significant ($p<0.01$) only in males at 6000 ppm, a dose which was shown to exceed the maximum tolerated by males by the high mortality it caused. The thyroid adenoma was likely a secondary effect of the thyroid-pituitary hormonal imbalance induced by chronic compound treatment. The increased incidences of neoplasms in the spleen and adrenal medulla were not dose-related and were of uncertain biological significance (spleen mononuclear cell leukemia in 75 and 200 ppm males and in 75, 200, and 1200 ppm females and adrenal medulla pheochromocytoma in 75, 200, and 1200 ppm males). There were also several neoplasms which were statistically elevated but incidental to treatment (skin papilloma in 75 ppm males, pituitary adenoma in 200 ppm males and mammary gland fibroadenoma in 1200 ppm females). Based on the significant depressions in mean body weights and mean net body weight gains in the rats, it appears that the maximum tolerated dose (MTD) was achieved in the study for both males (1200 ppm or 54.4 mg/kg/day) and females (6000 ppm or 334.7 mg/kg/day).

DATA EVALUATION REPORT

(11531

THIOPHANATE METHYL

Study Type: CHRONIC FEEDING/ONCOGENICITY– RAT (83-5)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Biomedical and Environmental Information Analysis Section
Health Sciences Research Division
Oak Ridge National Laboratory*
Oak Ridge, TN 37831
Task Order No. 94-34A

Primary Reviewer:
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Signature: S. Milanez
Date: 3-23-95

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Robert H. Ross, M.S., Group Leader

Signature: RHR
Date: 3/23/95

Quality Assurance:
Susan Chang, M.S.

Signature: Susan Chang
Date: 3/23/95

Disclaimer

The final Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Oak Ridge National Laboratory personnel.

*Managed by Martin Marietta Energy Systems, Inc., for the U.S. Department of Energy under Contract No. DE-AC05-84OR21400

EPA Reviewer: P. Hurley, Ph.D. Pamela M Hurley Date: 4/11/95
Review Section I, Toxicology Branch I (7509C)
EPA Section Head:M. Copley, Ph.D., D.A.B.T. Marion Copley Date: 4/17/95
Review Section I, Toxicology Branch I (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Chronic Feeding/Oncogenicity– Rat (83-5)

TOX. CHEM. NO: 375A

P.C.CODE.: 102001

MRID NO.: 428966-01

TEST MATERIAL: Thiophanate methyl

SYNONYMS: Thiophanate-methyl, Topsin-M, dimethyl-4,4'-(o-phenylene)-bis(3-thioallo phanate), dimethyl [1,2-phenylene-bis(iminocarbonothioyl)]-bis(carbamate)

STUDY NUMBER: 0566

SPONSOR: Nippon Soda Co., LTD., 2-1, 2-Chome, Ohtemachi, Chiyodaku, Tokyo, Japan

TESTING FACILITY: Toxicology Institute, Environmental Toxicology Laboratory, Nippon Soda Co., LTD., 345 Takada, Odawara, Kanagawa, Japan

TITLE OF REPORT: Thiophanate-methyl - Combined Chronic Toxicity/Oncogenicity Study in Rats

AUTHOR: H. Takaori

REPORT ISSUED: August 17, 1993

EXECUTIVE SUMMARY: In a 2-year feeding/oncogenicity study, thiophanate methyl was administered in the diet to 60 male and 60 female F344 rats/group at 0, 75, 200, 1200, or 6000 ppm. After week 52, 10 rats/sex/dose were sacrificed, except only five 6000 ppm males were sacrificed because 8 males died from non-treatment related injury at weeks 11 and 12. The mean compound consumption for the study was 0, 3.3, 8.8, 54.4, and 280.6 mg/kg/day for males and 0, 3.8, 10.2, 63.5, and 334.7 mg/kg/day for females.

Rats fed 75 and 200 ppm had no significant treatment-related toxic effects. By study termination, males had significantly decreased mean body weights in the 1200 ppm group (84% of controls, $p<0.001$) and in the 6000 ppm group (2 surviving males, 73% of controls (no statistics)). The mean net bodyweight gains were 79% of controls ($p<0.001$) for the 1200 ppm group and 63% of controls for the high dose group. Female rats had significant body weight changes only in the 6000 ppm dose group, the mean weight was 78% ($p<0.001$) and the mean net gain was 69% ($p<0.001$) of controls at the end of the study. Food efficiency in rats fed 1200 ppm was reduced to 78% and 88% of controls in males and females, respectively, while in 6000 ppm rats, the efficiencies were lowered to 65% and 71% in males and females. There was a treatment-related decrease in survival in only the 6000 ppm group males (2/55 survivors vs. 37/50 controls, $p<0.001$); the marginal increase in mortality ($p<0.05$) in the 200 ppm group males appeared spurious. Non-neoplastic pathological changes were observed primarily in 1200 and 6000 ppm rats in the liver (dose-related weight increase and hepatocellular hypertrophy), kidney (surface changes, dose-related increase in weight and severity of nephropathy), and thyroid (dose-related weight increase, follicular cell hypertrophy and hyperplasia, and T_3 and T_4 hormone level decreases). The levels of thyroid stimulating hormone (TSH) were elevated, though pituitary weights were unchanged. **A LOEL of 1200 ppm was identified for both male (54.4 mg/kg/day) and female (63.5 mg/kg/day) rats, based on treatment-related effects in the liver, kidneys, and thyroid. The corresponding NOEL was 200 ppm in both sexes of rats (corresponding to 8.8 mg/kg/day for males and 10.2 mg/kg/day for females), based on lack of significant toxic effects at this dose.**

The effects observed in the thyroid in 1200 and 6000 ppm male and female rats were accompanied by a dose-related increase in the incidence of follicular cell adenoma (males: 1/50, 0/48, 0/50, 3/50, 12/55 and females: 0/50, 0/49, 0/50, 1/50, 2/50 for doses of 0, 75, 200, 1200, and 6000 ppm, respectively; statistically significant ($p<0.01$) in males at 6000 ppm. There were statistically significant but non-dose related increases in the incidences of neoplasms in the spleen and adrenal medulla which were of uncertain biological significance (spleen mononuclear cell leukemia in 75 and 200 ppm males and in 75, 200, and 1200 ppm females and adrenal medulla pheochromocytoma in 75, 200, and 1200 ppm males). Based on the significant depressions in mean body weights and mean net body weight gains in both sexes and on the high mortality rate in males, it appears that the maximum tolerated dose (MTD) was exceeded at the high dose in males but achieved for males at 1200 ppm or 54.4 mg/kg/day and for females at 6000 ppm or 334.7 mg/kg/day.

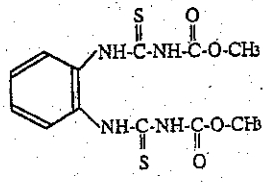
This study is classified as core guideline and satisfies the requirements for an 83-5 Chronic Feeding/ Oncogenicity Study in rats.

Special Review Criteria (40 CFR 154.7) None

A. MATERIALS

1. Test material: Thiophanate methyl

Description: pale brown powder
Lot/Batch No.: TIF-1016
Purity: 96.55% a.i.
Stability of compound:Stable at room temperature (not specified for how long)
CAS No.: 23564-05-8
Structure:



2. Vehicle and/or positive control

The test material was ground and mixed into the basal diet. No vehicle or positive control was included.

3. Test animals

Species: Rat
Strain: Fischer 344
Age and weight at study initiation: Rats were 6 weeks old. Weight, males: 109.9-132.7 g, females: 92.9-106.8 g.
Source: Charles River Japan, Inc., Atugi, Kanagawa, Japan
Housing: Rats were housed individually in stainless steel mesh cages. Males were moved to larger cages at 11 weeks.
Environmental conditions:
Temperature: 20.0-27.2°C
Humidity: 38.1%
Air changes: 10 times/hour
Photoperiod: 12 hours light, 12 hours darkness
Acclimation period: one week

B. STUDY DESIGN

1. Animal assignment

Animals were randomly assigned to the test groups in Table 1 by computer, with the criteria that the groups have similar values for mean weights and standard deviations.

TABLE 1. STUDY DESIGN

Dose in diet	Doses: Dosage achieved[a] (Mean) (mg/kg/day)		Number of Animals[b]: Main study (104 weeks)		Number of Animals[b]: Interim sacrifice (52 weeks)	
	Male	Female	Male	Female	Male	Female
0 ppm	0	0	50	50	10	10
75 ppm	3.3	3.8	50	50	10	10
200 ppm	8.8	10.2	50	50	10	10
1200 ppm	54.4	63.5	50	50	10	10
6000 ppm	280.6	334.7	55[c]	50	5[c]	10

[a]Doses were calculated by study authors using food consumption and body weight data and nominal concentrations, and are presented in Text Table IV, p. 28, MRID No. 428966-01.
[b]Data was taken from MRID No. 428966-01 (e.g. p. 12).
[c]In the 6000 ppm group males, fifty animals were intended to be in the main study group and 10 in the interim sacrifice group at the beginning of the study. Due to non-treatment related loss of 8 males at weeks 11 and 12, only 5 males were sacrificed after 52 weeks and the other 55 were considered by the authors to be part of the main study group.

Dose selection rationale: Doses tested were based on the results of a previous subchronic (90-day) range-finding study in rats (Nishibe and Takaori, MRID No. 420017-01). In this study, toxicity occurred at doses of 2200 ppm and higher (4200, 6200, and 8200 ppm); concentrations and results of lower doses were not reported. Effects caused by thiophanate methyl included anemia, hepatocellular hypertrophy, nephropathy, and thyroid follicular hypertrophy and hyperplasia. No body weight depression was noted at the highest dose tested (8200 ppm). The authors thus estimated that 6000 ppm could be the maximum tolerated dose (MTD) for a 2-year study, and doses of 75, 200, 1200, and 6000 ppm were selected.

2. Diet preparation and analysis

The test diet was prepared monthly by mixing with a blender appropriate amounts of ground test substance with the basal diet. The mixture was stored at -20°C until use. Homogeneity and concentration were tested for all doses of each monthly preparation, samples being taken at the top, middle, and

bottom position of each lot. The stability of thiophanate methyl in the formulated diet was confirmed prior to initiating the subchronic study by Nippon Soda Co., LTD. The stability, homogeneity, and concentration were tested by HPLC analysis.

Results –

a. Homogeneity/Concentration analysis – Each dietary concentration (75, 200, 1200, and 6000 ppm), at each sample position tested (top, middle, and bottom), had a mean analytical concentration which was from 94.1% to 110.9% of the target value.

b. Stability analysis – HPLC analysis showed that thiophanate methyl was stable for up to 7 days at room temperature (using 100 ppm), and for up to 58 days in a freezer at -20°C (using 75 ppm). The concentration of test compound determined by HPLC in the diet (mean of 3 measurements) was within 3% of the theoretical concentration at time 0 at each assay time.

3. Diet

Animals received food (basal diet M, Oriental Yeast Co., Ltd., Tokyo) and tap water *ad libitum.*

4. Statistics

The following statistical tests were used to analyze the results:
Chi-square test[1]:
mortality, clinical observation, ophthalmoscopic examination, macroscopic observation data, microscopic observations (neoplastic lesions)
Bartlett's test for homogeneity of variances[2]:
body weights, food consumption values, clinical laboratory measurements, absolute and relative organ weights
Mann-Whitney U-test[1]:
graded urinalysis data, microscopic observations (non-neoplastic lesions)

[1]The Fisher's exact test (two-tailed) was used when the number of animals was below 5/group
[2]If the variances were equal, parametric procedures were used (standard one-way ANOVA). For nonparametric procedures, the Kruskal-Wallis test was used. For both procedures, Dunnett's test or Scheffe's test were used to identify means which were significantly different from the control.

5. Signed and dated GLP and quality assurance statements were present.

C. METHODS AND RESULTS

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1. Observations

Animals were inspected at least once daily for signs of mortality, morbidity, and overt toxicity. Detailed clinical examinations were done weekly to assess gross pathological findings and palpable masses.

Results – Clinical observations that were statistically different from controls ($p < 0.05$ or $p < 0.01$) are shown in Table 2. Tissue masses in the subcutis and/or on the skin were found in both sexes of rats. Skin and mucous discoloration was found predominantly in males, while alopecia was dose-related and significantly elevated only in females. The incidence of tissue masses in the subcutis of 1200 ppm females was 2-fold greater in treated than control females from week 91 to 105, though the increase was statistically significant only at week 100. Significant elevations in the frequency of eyelid lacrimation were seen sporadically in 75, 200, and 1200 ppm females, while other eye effects (cornea, crystalline lens, skin) occurred in 200 ppm males, though these effects were likely incidental to compound treatment.

TABLE 2. TREATMENT-RELATED CLINICAL OBSERVATIONS IN RATS FED THIOPHANATE METHYL

Sex	Concentration (ppm)	Week(s) of observation	Clinical observations
Male	1200	95-101	Tissue masses in the subcutis, including in the lip
Male	1200, 6000	91 and/or 92	Tissue masses on the skin
Male	6000	77 to study termination (most weeks)	Pale discoloration of skin and mucous membranes
Female	1200	100	Tissue mass in the subcutis
Female	6000	79-104	Alopecia

Data was taken from Tables 1 and 2 (pages 49-122), MRID No. 428966-01.

Statistically significant increases in the mortality of males were seen at several weekly timepoints with lower doses: in the 200 ppm group at week 79 and at most weeks between weeks 94-105, and in the 1200 ppm group only at week 105. In the 6000 ppm male dose group, statistically significant elevations in mortality occurred from week 11 through study termination (105 weeks). Eight males of this group were killed in extremis or found dead at Weeks 11 or 12,

although their deaths were due to fracture of the nasal bone, considered by the authors to be due to problems with the cage feeder plate. The authors did not prepare Kaplan-Meier survival curves or in any other way censor the 8 treatment-unrelated deaths in 6000 ppm males. Among the female groups, treatment had no effect on mortality at any time. The mortality data is summarized in Text Table I, p. 26, MRID No. 428966-01, and is reproduced below (the total number of females at week 80 at 6000 ppm was changed from 60 to 50, correcting a typographical error) as Table 3.

TABLE 3. MORTALITY OF RATS FED THIOPHANATE METHYL 104 WEEKS

	Male			Female		
Dose (ppm)	wk 52	wk 80	wk 104	wk 52	wk 80	wk 104
0	0/60(0)	2/50(4)	13/50(26)	0/60(0)	3/50(6)	13/50(26)
75	0/60(0)	2/50(4)	15/50(30)	0/60(0)	1/50(2)	12/50(24)
200	0/60(0)	8/50(16)	24/50(48)a	0/60(0)	1/50(2)	8/50(16)
1200	0/60(0)	3/50(6)	21/50(42)	0/60(0)	0/50(0)	12/50(24)
6000	8/60(13)a	18/55(33)c	53/55(96)c	1/60(2)	3/50(6)	11/50(22)

Significantly different from control, a: $p<0.05$, c: $p<0.001$ (Chi-square test)

Data was taken from Text Table I, p. 26, MRID No. 428966-01A. The total number of females at week 80 at 6000 ppm was changed from 60 to 50, presumably correcting a typographical error.

2. Body weight

Animals were weighed once each week through treatment week 14, then at week 16, and once every 4 weeks thereafter.

Results – The mean body weight of male rats was statistically significantly lower than that of controls from week 84-104 in the 1200 ppm dose group and from week 52-104 in the 6000 ppm dose group. A biologically significant weight decrease (>10%) was first seen during weeks 100 and 68 in the 1200 and 6000 ppm males, respectively. The mean weights at 104 weeks were 84% (1200 ppm) and 73% (6000 ppm) of controls. Among females, statistically significant depressions in mean body weight occurred from weeks 20-52 and at week 88 in the 1200 ppm group, and from week 2-104 in the 6000 ppm group. The decreased mean weights of 1200 ppm females were not biologically significant at any week examined during the study, the weight being 91% of controls at week 104. The weight of 6000 ppm females fell below 90% of controls starting at week 48, and was 78% of controls at week 104. The decrease in mean body weights in both male and female rats was dose- and time-dependent throughout the study. Results are summarized in Text Table II, p. 27, MRID No. 428966-01, and are presented below as Table 4. The group mean body weight curves are presented in Figures 1 and 2 (p. 444 and 445) of MRID No. 428966-01.

TABLE 4. MEAN BODY WEIGHTS (g) AND RATIOS TO CONTROL (%)

	Male				Female			
Dose (ppm)	Wk 13	Wk 52	Wk 80	Wk 104	Wk 13	Wk 52	Wk 80	Wk 104
0	353.8 (100)	471.9 (100)	484.5 (100)	445.6 (100)	193.6 (100)	258.1 (100)	306.7 (100)	327.9 (100)
75	353.2 (100)	473.7 (100)	480.8 (99)	432.4 (97)	194.5 (100)	263.8 (102)	312.3 (102)	327.5 (100)
200	354.7 (100)	478.4 (101)	481.5 (99)	436.9 (98)	194.1 (100)	258.8 (100)	305.7 (100)	316.6 (97)
1200	350.6 (99)	469.2 (99)	467.1 (96)	376.3 (84)c	190.3 (98)	248.4 (96)b	291.4 (95)	299.6 (91)
6000	353.6 (100)	451.4 (96)b	402.3 (83)c	323.1 (73)d	182.5 (94)b	227.4 (88)c	251.4 (82)b	256.4 (78)c

Significantly different from control, b:p<0.01, c:p<0.001 (Chi-square test)
d: Statistical analysis was not performed due to the small number of surviving animals

Data was taken from Text Table II, p. 27, MRID No. 428966-01A

The net body weight gains were depressed in both the male and female rats treated with 1200 and 6000 ppm thiophanate methyl. At the end of the study, the net gain of the 1200 ppm and 6000 ppm males were 79% and 63%, respectively, of the control males. In the female 1200 and 6000 ppm dose groups, the net gains were 88% and 69%, respectively, of the controls at week 104. There was a dose-related decrease in mean net weight gain in both sexes of rats throughout the study. The pattern of weight gain during the study is presented in Text Table II, p. 27, MRID No. 428966-01, and is shown below as Table 5. Based on the >10% depression of body weight gain at study termination in rats treated with 1200 ppm, the study authors propose this concentration as the MTD in both males and females. The reviewer, however, feels that for the females, 6000 ppm is the MTD because the mean body weight of the animals was depressed by only 9% at the end of the study (see Table 4 above), which was not biologically or statistically significant.

TABLE 5. MEAN NET WEIGHT GAINS (g) AND RATIOS TO CONTROL (%)

	Male				Female			
Dose (ppm)	Wk 13	Wk 52	Wk 80	Wk 104	wk 13	Wk 52	Wk 80	Wk 104
0	232.7 (100)	350.8 (100)	362.8 (100)	324.8 (100)	93.9 (100)	158.5 (100)	206.9 (100)	227.8 (100)
75	232.1 (100)	352.5 (100)	359.8 (99)	312.3 (96)	94.8 (101)	164.1 (104)	212.8 (103)	227.9 (100)
200	233.4 (100)	355.2 (101)	360.1 (99)	315.3 (97)	94.4 (101)	159.1 (100)	206.1 (100)	216.9 (95)
1200	229.4 (99)	348.1 (99)	345.6 (95)	256.7 (79)c	90.6 (96)	148.7 (94)b	191.5 (93)	199.4 (88)
6000	232.4 (100)	330.2 (94)b	280.7 (77)c	204.5 (63)d	82.8 (88)b	127.7 (81)c	151.4 (73)b	156.5 (69)c

Significantly different from control, b:p<0.01, c:p<0.001 (Chi-square test)
d: Statistical analysis was not performed due to the small number of surviving animals

Data was taken from Text Table II, p. 27, MRID No. 428966-01A,

3. Food consumption and compound intake

Food consumption for each animal was determined once each week for a 24-hour period through treatment week 14, then at week 16, and once every 4 weeks thereafter. Mean daily diet consumption was calculated as g food/animal/day and as g food/kg body weight/day. Only selected food efficiency values ([body weight gain (g/day) /food consumed (g/day)] X 100) were calculated by study authors. Compound intake (mg/kg/day) values were calculated as time-weighted averages from the food consumption and body weight data.

Results –

a. Food consumption – Calculated as g/animal/day, food consumption was statistically significantly altered (increased or decreased) occasionally throughout the study in both sexes. The changes were small (usually < 10%) and not related to dose. The 6000 ppm groups had fairly consistent increases (generally <20%) in food consumption/body weight starting at week 32 in males and starting at week 5 in females, through the remainder of the study. The food consumption/ kg body weight/ day was increased or decreased during several periods of the first year for both males and females at various doses, while during the second year, values for both sexes fed 6000 ppm were increased at almost every time point examined. Overall, there was a dose-related increase in the g food/kg body weight/day. Results are summarized in Table 6. The mean daily food consumption (g/animal/day) for the rats and are presented graphically in Figures 3 and 4 (p. 446 and 447) of MRID No. 428966-01.

TABLE 6. MEAN DAILY FOOD CONSUMPTION AND FOOD EFFICIENCY OF RATS FED THIOPHANATE METHYL IN THE DIET FOR 104 WEEKS

	Food Consumption				Food efficiency[a]	
	g/animal/day		g/kg body weight/day			
Dose	Male	Female	Male	Female	Male	Female
0 ppm	18.8	12.9	43.9	50.6	2.3	2.4
75 ppm	18.9	12.8	44.3	50.0	2.2	2.4
200 ppm	18.9	12.8	44.1	50.9	2.2	2.3
1200 ppm	18.9	12.8	45.3	52.9	1.8	2.1
6000 ppm	18.4	12.4	46.8	55.8	1.5	1.7

Data was taken from Text Table IV, p. 28, MRID No. 428966-01.
[a]The food efficiencies were calculated by the reviewer using the mean net weight gain data from Text Table III, p. 27, MRID No. 428966-01 and the food consumption data (g/animal/day) from this table.

b. Compound consumption (time-weighted average) – The group mean values over the 104-week study period for the 0, 75, 200, 1200, and 6000 ppm animals were 0, 3.3, 8.8, 54.4, 280.6 mg/kg/day for males and 0, 3.8, 10.2, 63.5, and 334.7 mg/kg/day for females.

c. Food efficiency – The authors calculated food efficiencies for selected weekly intervals (weekly through treatment week 14, then at week 16, and once every 4 weeks thereafter); the values for the entire study (weeks 1-104) were calculated by the reviewer by dividing the total body weight gained by the total mean food consumption (consumption/day X 728 days (104 weeks)) X 100. There was a dose-related decrease in the food efficiency in both sexes of rats, values ranging from 63% to 96% of controls for the males and from 71% to 101% of controls for females (high-dose to low-dose groups). The relatively minor changes in total food consumption coupled with the marked decrease in total body weight gain suggest that the decreased efficiencies are due to compound consumption. The results are presented in Table 6.

4. Ophthalmoscopic examination

Eyes of all animals were examined with the direct ophthalmoscope and the fundus camera prior to study initiation and at months 12 and 24. All animals from the control and highest dose groups (6000 ppm) were also examined at 6 and 18 months.

Results– All the rats chosen for the study had normal ophthalmoscopic results (7 rats that had abnormalities were excluded from the study). No treatment-related lesions were found in any group at any of the times examined.

5. Blood was collected from the retro-orbital sinus of 10 rats/sex/dose group for hematology and clinical chemistry analysis (except from only one male of the 6000 ppm group at month 24). Only 8 rats/sex/group were analyzed for thyroid hormones (T_3 and T_4) and TSH, while no 6000 ppm males were examined at month 24. Collection of blood for hematology analysis (no fasting) was at months 3, 6, 12, 18, and 24 of the study. Animals were fasted for 16 hours prior to withdrawal of blood for blood chemistry analysis at months 6, 12, 18, and 24. Slides for examining differential leukocyte counts were prepared at all blood collection times for the control and high-dose groups, and at months 12, 18, and 24 for the intermediate dose groups (75, 200, 1200 ppm). The CHECKED (X) parameters were examined.

a. Hematology

X			X	
X	Hematocrit(HCT)*		X	Leukocyte differential count*
X	Hemoglobin (HGB)*		X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*		X	Mean corpusc. HGB conc. (MCHC)
X	Erythrocyte count (RBC)*		X	Mean corpusc. volume (MCV)
X	Platelet count*			Reticulocyte count
	Blood clotting measurements			
	(Thromboplastin time)			
	(Clotting time)			
	(Prothrombin time)			

* Required for subchronic and chronic studies.

Results – In 6000 ppm males, statistically significant decreases were seen in the hematocrit (4%-17% at months 3-18), hemoglobin levels (6%-21% at months 3-18), MCH (4%-8% at months 3-18), the RBC count (8%,14% at months 12,18), the MCV (2% at months 3,12), and the MCHC (2%, 5% at months 3, 18). Increases were seen in the platelet count (5%-48% at months 3-18) and in white blood cells (WBC) (14%-63% at months 6-18). In 1200 ppm males, a 5% decrease in hemoglobin levels occurred at 12 months. Females treated with 6000 ppm thiophanate methyl had statistically significantly decreased hematocrit (7%, 5% at months 6,12), hemoglobin (5%-7% at months 3-12), MCV (3%-6% at months 3-18), MCH (5%-9% at months 3-12) and MCHC (2% at 3 months). At 1200 ppm, females had lowered MCV (3% at month 12) and MCH (2%, 4% at months 3, 12). The number of platelets was increased only at 6 months (13%) in 6000 pm females, whereas the WBC was unaffected. There were no significant changes in the differential leukocyte counts at any dose tested in either sex. In both male and female rats, the severity of the observed effects increased with time, though a clear dose-response for the various parameters was not usually seen. The persistent and time-dependent changes in the hematocrit, hemoglobin, MCH, platelet and WBC counts at 6000 ppm suggests these effects (constituting a mild anemia) were treatment-related, though the small magnitude of the changes relative to controls indicates they were not biologically significant in either sex.

b. Clinical chemistry

X	Electrolytes:
X	Calcium*
X	Chloride*
	Magnesium
X	Phosphorus*
X	Potassium*
X	Sodium*

	Enzymes
X	Alkaline phosphatase (ALK)
X	Cholinesterase (ChE)
X	Creatinine phosphokinase (CPK)
X	Lactic acid dehydrogenase (LDH)
X	Serum alanine aminotransferase (also SGPT)*
X	Serum aspartate aminotransferase (also SGOT)*
	Gamma glutamyl transferase (GGT)
	Glutamate dehydrogenase
X	Triiodothyronine (T_3)
X	Thyroxine (T_4)
X	Thyroid stimulating hormone (TSH)

X	Other:
X	Albumin*
X	Blood creatinine*
X	Blood urea nitrogen*
X	Cholesterol*
	Globulins
X	Glucose*
X	Total bilirubin
X	Total serum protein (TP)*
	Triglycerides
	Serum protein electrophoresis

* Required for subchronic and chronic studies.

Results – The most marked clinical chemistry change was the increase in the total serum cholesterol at 1200 and 6000 ppm, which was dose- and time-dependent (6-24 months) in both sexes of rats. The albumin/globulin (A/G) ratio was depressed in 6000 ppm males at 6, 12, 18 and 24 months ($p<0.01$, except at 24 months there was no statistical analysis), and in 1200 ppm males at 24 months ($p>0.01$). In 6000 ppm females, the A/G ratio was depressed at 6 and 24-months ($p<0.01$). The A/G ratio was generally dose- and time-dependent in both sexes. The blood urea nitrogen (BUN) was increased in 6000 ppm males at 12 and 18 months ($p<0.01$), while at 24 months the BUN was increased by >50% in all dose groups. Males (1200 and 6000 ppm) also had increased serum creatinine (about 1.5-fold) and somewhat decreased serum albumin (<30%) at 12 months or later, the changes in both parameters were typically greater at higher doses. Other parameters were altered in both sexes of rats (total protein, electrolytes, enzyme levels) though they were not considered to be treatment-related because they only occurred at 6 and/or 12 months, or the changes were not biologically significant (<10% different from controls). No changes were noted in sodium, calcium, alkaline phosphatase, or glucose. Table 7 presents the significant clinical chemistry changes in males and females.

TABLE 7. CLINICAL CHEMISTRY PARAMETERS IN THIOPHANATE METHYL TREATED AND CONTROL RATS

Parameter	Dose (ppm)	Month			
		6	12	18	24
Males					
Total cholesterol	0	80.3	111.0	159.9	239.8
	75	84.3	110.3	137.9	275.1
	200	89.6	134.4	156.6	332.6
	1200	100.7**	142.7	195.5	389.1*
	6000	192.8**	285.0**	330.5**	405.8 (NA)
BUN	0	17.9	21.1	19.5	19.4
	75	17.9	20.3	19.3	32.4**
	200	17.7	21.4	18.2	29.3*
	1200	16.3	21.6	20.7	38.6**
	6000	18.5	26.5**	44.5**	51.2 (NA)
Creatinine	0	0.77	0.65	0.67	0.85
	75	0.74	0.65	0.64	1.05
	200	0.87	0.63	0.78	1.07
	1200	0.83	0.69	0.80	1.34**
	6000	0.85	0.73	1.20*	1.42 (NA)
Albumin	0	4.7	4.5	4.1	3.7
	75	4.8	4.5	4.0	3.2
	200	4.8	4.5	4.0	3.4
	1200	4.9	4.3	3.7*	2.7**
	6000	4.6	3.8**	3.2**	3.4 (NA)
A/G ratio	0	1.35	1.34	1.22	1.02
	75	1.33	1.37	1.16	0.86
	200	1.34	1.31	1.10	0.85
	1200	1.31	1.21	1.01	0.69 **
	6000	1.12**	0.92**	0.78**	0.72 (NA)
Females					
Total cholesterol	0	102.7	129.6	137.6	150.8
	75	103.3	134.6	136.6	170.5
	200	107.9	138.5	131.9	160.0
	1200	124.0**	170.2**	155.3	209.8
	6000	204.2**	247.1**	247.9**	366.6**
A/G ratio	0	1.52	1.74	1.48	1.42
	75	1.52	1.68	1.48	1.48
	200	1.48	1.65	1.32	1.41
	1200	1.41	1.62	1.49	1.27
	6000	1.31**	1.45	1.26	1.07**

Data was taken from Tables 19 and 20, MRID No. 428966-01.
Significantly different from control: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$,
NA = No statistical analysis performed because only one animal was examined.

Minor alterations in serum cholinesterase activity occurred in male and female rats. In males, there was most commonly an increase in the levels, which was statistically significant ($p<0.05$) at 6 and 12 months (6000 ppm), though not biologically relevant. The > 10% decreases in males at 18 months (75 ppm) and 24 months (6000 ppm) were spurious and not statistically significant. In females, there was a transient, dose-related decrease in serum cholinesterase levels at 6 and 12 months, while at 18 and 24 months, only slight decreases (<10%) were seen at all doses except 1200 ppm. The effects in the females did not appear to be biologically significant, as there were no associated clinical (neurological) effects. Results for males and females are presented in Table 8.

TABLE 8. SERUM CHOLINESTERASE LEVELS IN THIOPHANATE METHYL TREATED AND CONTROL RATS

Dose (ppm)	Males (month)				Females (month)			
	6	12	18	24	6	12	18	24
0	556	1072	1302	2280	4071	4741	3557	3961
75	527 (95)[a]	1273 (119)	1027 (79)	2141 (94)	3922 (96)	4794 (101)	3723 (105)	4055 (102)
200	595 (107)	1330 (124)	1214 (93)	2662 (117)	3639 (90)	4596 (97)	3459 (97)	3819 (96)
1200	598 (198)	1251 (117)	1344 (103)	2072 (91)	3274* (81)	3878**(82)	2915 (82)	3118*(79)
6000	792*(142)	1512**(141)	1524 (117)	[1413][b](62)	2629**(65)	3793**(80)	3326 (94)	3758 (95)

Data was taken from Tables 19 and 20 (pages 202-234), No. 428966-01.
[a]Numbers in parentheses are the % of controls values for the individual time points.
[b]Number in brackets was not analyzed statistically due to inadequate number of animals.
Significantly different from control, * $p< 0.05$; ** $p< 0.01$

The levels of the thyroid hormones T_3 and T_4 were significantly lowered primarily in 6000 ppm males throughout the 2-year study. The levels generally decreased with time, though a clear dose-response was not observed. At 6000 ppm, T_3 levels were 86% ($p<0.05$) and 74% ($p<0.01$) of controls at months 12 and 18, respectively; 6000 ppm males were not examined at 24 months. At 1200 ppm, male T_3 levels were significantly altered only at 24 months, being 76% ($p<0.05$) of controls. T_4 levels were depressed at months 6, 12, and 18 in 6000 ppm males (being 66%, 80%, and 45% of controls, respectively, $p< 0.01$), and at month 24 in 1200 ppm males (55% of controls, $p<0.05$). TSH was elevated 1.5 to 2-fold in 6000 ppm males at months 6, 12, and 18 ($p<0.01$), and a modest increase (22%, $p<0.05$) was seen at 18 months in the

1200 ppm group. Female thyroid hormone levels were changed less than that of the males: significant changes in T_3 levels were not observed, whereas T_4 was decreased only at month 18 (73% of controls at 6000 ppm, $p<0.01$). TSH was increased 1.4 to 1.8-fold at months 12, 18, and 24 in 6000 ppm females (no time-dependency; $p<0.01$ at 12 and 18 months, $p>0.05$ at 24 months). Results for males and females are presented in Table 9.

TABLE 9. HORMONE LEVELS IN THIOPHANATE METHYL TREATED AND CONTROL RATS

Dose (ppm)	Males (month)				Females (month)			
	6	12	18	24	6	12	18	24
Triiodothyronine (T_3)								
0	0.915	0.913	0.776	0.791	0.975	0.828	0.785	0.830
75	0.990 (108)	0.821 (90)	0.793 (102)	0.755 (95)	0.964 (99)	0.796 (96)	0.780 (99)	0.800 (96)
200	0.905 (99)	0.850 (93)	0.775 (100)	0.776 (98)	0.860 (88)	0.899 (109)	0.796 (101)	0.870 (105)
1200	1.031 (113)	0.916 (100)	0.764 (98)	0.600* (76)	0.843 (86)	0.989 (119)	0.918 (117)	0.949 (114)
6000	0.801 (88)	0.783* (86)	0.573**(74)	NE	0.894 (92)	0.993 (120)	0.845 (108)	0.935 (113)
Thyroxine (T_4)								
0	7.08	5.89	5.38	2.71	4.65	3.94	4.70	2.84
75	6.64 (94)	5.29 (90)	4.95 (92)	2.58 (95)	4.73 (102)	3.38 (86)	4.13 (88)	2.91 (102)
200	6.35 (90)	5.41 (92)	5.48 (102)	2.46 (91)	4.09 (88)	3.81 (97)	4.06 (86)	2.71 (95)
1200	6.40 (90)	5.61 (95)	4.93 (92)	1.48* (55)	4.00 (86)	4.24 (108)	4.13 (88)	3.05 (107)
6000	4.70** (66)	4.70** (80)	2.41** (45)	NE	4.61 (99)	4.84 (123)	3.41** (73)	2.34 (82)
Thyroid stimulating hormone (TSH)								
0	0.605	0.645	0.709	0.774	1.021	0.473	1.116	0.595
75	0.701 (116)	0.616 (96)	0.793 (112)	1.175 (152)	1.098 (108)	0.486 (103)	1.216 (109)	0.604 (102)
200	0.733 (121)	0.791 (123)	0.870 (123)	0.863 (111)	1.181 (116)	0.495 (105)	1.238 (111)	0.576 (97)
1200	0.836 (138)	0.768 (119)	0.886* (122)	0.841 (109)	1.076 (105)	0.509 (108)	1.266 (113)	0.591 (99)
6000	1.309**(216)	1.083** (168)	1.044***(147)	NE	1.298 (127)	0.756**(160)	2.004** (180)	0.813* (137)

Data was taken from Tables 19 and 20 (pages 202-234), MRID No. 428966-01.
* Significantly different from controls: * $p< 0.05$, ** $p< 0.01$, *** $p< 0.001$
NE = Not examined
*Numbers in parentheses are the % of controls values for the individual time points.

Additional experiments performed and included along with this study examined the mechanism of the effect of thiophanate methyl on the thyroid (and liver) are presented in Annex 5 (pages 529-535) of MRID No. 428966-01, and are summarized on Appendix pages A-1 and A-2.

6. Urinalysis

Urine was collected from 10 fasted rats/sex/group at months 6, 12, 18, and 24 (only one male at month 24). Urine sediment was analyzed from 5 rats/sex/group at 6 months, and from 10 rats/sex/group at 12, 18, and 24 months. Because the 18 month urinary protein levels exceeded the upper limit of detectability in the original protein assay used (CLINITEK 100), an alternate method (nephelometry) was also used for all time points (with frozen urine). Water consumption was measured at months 18 and 24 because an increase in the urinary volume of 6000 ppm males was noticed at 12 months. The CHECKED (X) parameters were examined.

X		X	
X	Appearance*	X	Glucose*
X	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*		Nitrate
X	Protein*	X	Urobilinogen

* Required for chronic studies.

Results - Few treatment-related changes were seen in urinalysis parameters, primarily occurring in males fed 6000 ppm thiophanate methyl. Significant, dose-related increases (3 to 7-fold) in urinary protein were seen in 6000 ppm males at 6 months ($p<0.01$), 12 months ($p<0.05$), and 18 months ($p<0.01$). The one 6000 ppm male at 24 months had a 3-fold increase in total protein. In males fed 1200 ppm, urinary protein was elevated 2.5 ($p<0.05$) and 3-fold ($p<0.01$), respectively, at months 12 and 24. The 2-fold increase ($p<0.05$) in urinary protein in 200 ppm males at month 24 was likely not biologically significant, as no histological effects were seen in the kidneys at this dose. There were other parameters in 1200 and/or 6000 ppm males which had significantly different values than controls, and may have been secondary effects of the observed liver and kidney degeneration in the animals. These effects include increases in ketone bodies (6, 12 months), water consumption (18 months) and in urinary volume (12,18 months), and decreased urine pH (6,12, 18 months) and specific gravity (12, 18 months). Females treated with 6000 ppm thiophanate methyl had increases in urine protein (12 months) and

water consumption (18 months) which were incidental to treatment. Microscopic examination of the urine sediment of both sexes of rats showed no dose-related effects, though statistically significant changes were seen sporadically in the number of hyaline casts, triple phosphate crystals, and in the number of epithelial cells. The color, glucose content, bilirubin, occult blood, and urobilinogen were similar to that of controls at all test times.

7. Sacrifice and pathology

All animals that died spontaneously or were sacrificed in extremis or on schedule (52 or 104 weeks) were subject to a complete necropsy. Rats were fasted 16 hours prior to sacrifice and weighed. Animals killed in extremis were not fasted. Interim-sacrifice rats were killed by sodium pentobarbital anesthetization and exsanguination whereas animals sacrificed in extremis or after 104 weeks were killed by chloroform inhalation. The CHECKED (X) tissues were collected for histological examination. Organs and tissues were preserved and fixed in 10% buffered formaldehyde solution and paraffin sections were stained with hematoxylin and eosin. Histopathology was performed on all organs in all interim-sacrificed animals, controls, 6000 ppm males and females, 1200 ppm males, and animals that died or were killed during the study. In the other dose group animals, the lung, liver, kidney, thyroid, parathyroid, pituitary, adrenal, and all gross lesions were examined. The (XX) organs, in addition, were weighed.

X	Digestive system
	Tongue
x	Salivary glands*
x	Esophagus*
x	Stomach*
x	Duodenum*
x	Jejunum*
x	Ileum*
x	Cecum*
x	Colon*
x	Rectum*
xx	Liver*+
	Gall bladder*
x	Pancreas*

	Respiratory
x	Trachea*
xx	Lung*
	Nose
	Pharynx
	Larynx

X	Cardiovasc./Hemat.
x	Aorta*
xx	Heart*
x	Bone marrow*
x	Lymph nodes*
xx	Spleen
x	Thymus*

	Urogenital
xx	Kidneys*+
x	Urinary bladder*
xx	Testes*+
x	Epididymides
	Prostate
	Seminal vesicle
xx	Ovaries*+
x	Uterus*

X	Neurologic
xx	Brain*+
x	Periph. nerve*
x	Sinal cord (3 levels)*
xx	Pituitary*
x	Eyes (optic n.)*

	Glandular
xx	Adrenal gland*
	Lacrimal gland
x	Mammary gland*
xx	Parathyroids*++
xx	Thyroids*++

	Other
x	Bone*
x	Skeletal muscle*
x	Skin*
x	All gross lesions and masses*

* Required for subchronic and chronic studies.
+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.

Results –

a. Organ weight – Male and female rats treated with 1200 and/or 6000 ppm thiophanate methyl had statistically significant increases in both the absolute and relative weights of the thyroid, liver and kidney at 12 and 24 months. The weight increases were generally dose- and time-dependent in both sexes of rats. The greatest organ weight increase was seen in the thyroid in 6000 ppm rats. The relative and/or absolute thyroid weight was about 2-fold greater than of controls at 12 months in both sexes and at 24 months in females (the two 24-month surviving males had an even greater increase in thyroid weight). In the lungs and spleen, either the absolute and relative weights or only the relative weights were increased in various dose groups at 12 or 24 months. Only the relative weight was increased in the heart, adrenals, brain, and ovaries in 1200 and/or 6000 ppm treated groups. The weight changes of the heart, adrenals, brain, ovaries and lungs were probably not treatment-related since pertinent histological changes

were not seen in the organs at 1200 and/or 6000 ppm, and may be due to the depressions in overall body weights that were seen in the treated rats. The absolute and relative weight of the pituitary and testis were not affected in any group. Results for the thyroid, liver, kidney, and pituitary are presented in Table 10, the latter being included because an increase in TSH was observed in many animals.

TABLE 10. ABSOLUTE AND RELATIVE (%) MEAN ORGAN WEIGHTS (g) FOLLOWING TREATMENT OF RATS WITH THIOPHANATE METHYL

Dose (ppm)	Males - 12 Months				Females - 12 Months			
	Thyroid	Pituitary	Liver	Kidney (R)[a]	Thyroid	Pituitary	Liver	Kidney (R)[a]
0	0.024 (0.005)[b]	0.010 (0.002)	11.237 (2.564)	1.329 (0.303)	0.017 (0.007)	0.017 (0.007)	5.523 (2.203)	0.763 (0.307)
75	0.028 (0.006)	0.012 (0.003)	11.586 (2.646)	1.371 (0.313)	0.018 (0.007)	0.014 (0.005)	6.045 (2.352)	0.823* (0.321)
200	0.029 (0.006)	0.011 (0.002)	12.100 (2.673)	1.392 (0.308)	0.019 (0.008)	0.014 (0.006)	6.036 (2.462)	0.801 (0.328)
1200	0.033** (0.008)**	0.011 (0.003)	13.288** (3.067)**	1.412* (0.327)*	0.023** (0.010)**	0.014 (0.006)	6.403* (2.817)**	0.842** (0.374)**
6000	0.060*** (0.015)***	0.010 (0.002)	17.849*** (4.343)***	1.647*** (0.402)***	0.035** (0.016)**	0.016 (0.007)	8.123** (3.749)**	0.884** (0.408)**
	Males - 24 Months				Females - 24 Months			
0	0.033 (0.008)	0.036 (0.009)	12.841 (3.097)	1.623 (0.391)	0.022 (0.007)	0.057 (0.021)	7.685 (2.492)	1.061 (0.347)
75	0.034 (0.009)	0.025 (0.006)	13.454 (3.375)	1.696 (0.427)	0.027 (0.009)	0.025 (0.009)	8.039 (2.595)	1.059 (0.342)
200	0.071 (0.022)	0.036 (0.009)	13.811 (3.455)	1.752 (0.443)	0.033 (0.012)	0.030 (0.011)	8.271 (2.781)	1.093 (0.370)
1200	0.041*** (0.012)***	0.069 (0.022)	16.021*** (4.627)***	1.943*** (0.571)***	0.028** (0.010)***	0.025 (0.009)	9.548*** (3.439)***	1.148* (0.413)**
6000[c]	0.320 (0.103)	0.013 (0.004)	19.141 (6.451)	1.901 (0.635)	0.039*** (0.017)***	0.023 (0.010)	10.807*** (4.602)***	1.216*** (0.517)***

Data was taken from Tables 23-26 (pages 270-293), MRID No. 428966-01.
[a]Results for the left kidney were nearly identical as the results for the right kidney
[b]Numbers in parentheses are the relative organ weights (mean organ weight/body weight ratio (%))
[c]Statistical analysis was not performed for the males due to inadequate number of animals (2).
Significantly different from control: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

b. Gross pathology – At the interim (12 month) sacrifice, statistically significant incidences of pathologic changes occurred in the liver (brownish-black or brown) and kidney (granular and/or brownish black) in both male and female 6000 ppm rats. Some kidney discoloration was also seen at 1200 ppm in both sexes.

In the main study, animals examined after death (24-month sacrifice and unscheduled death) had numerous pathological lesions, the most significant treatment-related effects occurring in the kidneys and thyroid in both sexes. In males, there was a dose-related increase in kidney lesions (granular, pale brown) and in thyroid swelling, the increases being quite marked at the 6000 ppm dose ($p<0.001$). The nonsignificant increase in thyroid masses in 6000 ppm males was also likely treatment-related, as the thyroid was identified as a target organ for compound toxicity by clinical and histological analysis. The increased incidences of lesions in the heart, ventricles, and descending thoracic aorta (6000 ppm males) may have been a secondary effect of kidney degeneration, and were consistent with histological changes (calcification, fibrosis) seen in the hearts of males. There were spurious increases in subcutaneous tissue masses and submandibular lymph node swelling in males at 1200 ppm. The decreased number of masses in the testes (38/50 in controls vs. 24/50 at 200 ppm and 30/55 at 6000 ppm) and pituitary (16/50 in controls vs. 0/55 at 6000 ppm) were not biologically relevant. In females, significantly increased incidences of lesions occurred in the skin (alopecia), kidney (black, brownish black), and thyroid (swelling) at 6000 ppm. The incidences of the lesions in females generally increased with dose. The decrease in the number of females with pituitary masses (17/50 in controls vs. 6/50 at 6000 ppm) was incidental to treatment. The gross pathology results are summarized in Table 11.

TABLE 11. MACROSCOPIC CHANGES IN TERMINAL SACRIFICE AND UNSCHEDULED-DEATH RATS ADMINISTERED THIOPHANATE METHYL[a]

Organ	Morphology	Dose (ppm)				
		0	75	200	1200	6000
Males						
Subcutaneous tissue	Mass	4/50	12/50	8/50	14/50**	3/55
Heart	Area, white[b]	0/50	0/50	0/50	1/50	6/55*
Ventricle	Area, white[b]	0/50	2/50	4/50	3/50	8/55*
Descending thoracic aorta	Distended	1/50	1/50	0/50	2/50	11/55**
Submandibular lymph node	Swelling	3/50	4/50	6/50	9/50*	3/55
Kidney	Granular, pale brown[b]	3/50	6/50	6/50	17/50***	26/55***
Thyroid	Mass Swelling[b]	4/50 1/50	2/50 2/50	2/50 1/50	3/50 5/50	8/55 38/55***
Females						
Skin	Alopecia[b]	11/50	8/50	14/50	18/50	21/50*
Spleen	Swelling	5/50	9/50	5/50	12/50*	8/50
Kidney	Black[b] Brownish-black[b]	0/50 4/50	1/50 0/50	1/50 1/50	1/50 1/50	6/50* 12/50*
Thyroid	Mass Swelling[b]	0/50 0/50	2/50 1/50	2/50 3/50	1/50 1/50	1/50 15/50***

[a]Data was taken from Tables 27-30, MRID No. 428966-01, and is presented as the number of animals showing a lesion/number of animals examined. Statistical significance was calculated by the reviewer using the Fischer exact test. Significantly different from controls: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$
[b]Cochran-Armitage trend test indicated there was a dose-related response ($p < 0.05$) for the four tested doses.

c. Microscopic pathology –

1) Non-neoplastic – At the interim sacrifice (12 months), significant treatment-related non-neoplastic lesions were seen in the liver, thyroid, adrenal cortex, and kidney. The results are summarized in Table 12. Hepatocellular hypertrophy and deposition of lipofuscin pigment occurred in the liver in both male and female 1200 and 6000 ppm group rats. In the thyroid, follicular hypertrophy and hyperplasia were found in 1200 and 6000 ppm males and females. Focal hyperplasia of thyroid follicular cells occurred in two 6000 ppm females, and although not statistically significantly elevated, was probably treatment-related. There was a significant elevation of cytoplasmic

lipid in adrenal cortical cells in 1200 ppm females and in both sexes of 6000 ppm rats. Lipofuscin pigmentation and an increase in the severity of nephropathy occurred in the kidneys of male and female 6000 ppm rats. Several statistically significant changes were seen which were likely spontaneous events, including decreases in the severity of thyroid parafollicular cell hyperplasia and mesenteric lymph node microgranuloma in 1200 and/or 6000 ppm females, and an increase in thyroid calcium deposition in 75 ppm males.

TABLE 12. NONNEOPLASTIC LESIONS IN RATS ADMINISTERED THIOPHANATE METHYL AFTER 12 MONTHS (INTERIM SACRIFICE)[a]

Organs/Lesions	Doses (ppm)				
	0	75	200	1200	6000
Males					
Liver/Hypertrophy and Lipofuscin	0/10	0/10	0/10	10/10** (1.7)	5/5** (3.0)
Kidney/Nephropathy /Lipofuscin pigmentation	10/10 (2.0) 0/10	10/10 (1.9) 0/10	10/10 (2.0) 0/10	10/10 (2.5) 0/10	5/5** (3.0) 4/5* (1.0)
Thyroid/ Calcium deposition /Hypertrophy and Hyperplasia	2/10 (1.0) 0/10	8/10* (1.0) 0/10	2/10 (1.0) 0/10	5/10 (1.0) 10/10** (1.0)	1/5 (1.0) 5/5** (2.2)
Adrenal cortex/ Lipidosis	0/10	0/10	2/10 (1.0)	0/10	4/5* (1.0)
Females					
Mesenteric lymph node /Microgranuloma	10/10 (2.0)	10/10 (1.8)	10/10 (1.5)	9/10** (1.3)	10/10** (1.3)
Liver/ Hypertrophy and Lipofuscin	0/10	0/10	0/10	10/10** (1.1)	10/10** (2.0)
Kidney/ Nephropathy /Lipofuscin pigmentation	10/10 (1.0) 0/10	10/10 (1.0) 0/10	10/10 (1.1) 0/10	10/10 (1.1) 0/10	10/10** (1.9) 10/10** (1.0)
Thyroid/ Hyperplasia, focal /Hypertrophy and hyperplasia /Hyperplasia, parafollicular cell	0/10 0/10 10/10 (2.1)	0/10 0/10 10/10 (2.0)	0/10 0/10 10/10 (1.9)	0/10 5/10 (1.0) 10/10 (2.0)	2/10 (1.5) 10/10** (2.1) 10/10* (1.4)
Adrenal cortex/ Lipidosis	0/10	3/10 (1.0)	0/10	6/10* (1.0)	10/10* (1.0)

[a]Data was taken from Tables 31 and 32, MRID No. 428966-01, and is presented as the number of animals showing a lesion/number of animals examined. The numbers in parentheses are the average severity rating or grade: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
Significantly different from control: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$,

Histological analysis of the main study rats showed that the most significant treatment-related lesions occurred in the liver, kidney, and thyroid in both sexes of rats (see Tables 13 and 14). Liver hypertrophy and lipofuscin pigmentation and thyroid hypertrophy and hyperplasia were the most dramatically elevated in both males and females, being found in 0-2% of controls and in 84-98% of animals at 6000 ppm. The incidences of both lesions were dose-related. Other significant effects seen in the liver of males were focal fatty degeneration and focal necrosis at 6000 ppm, and multiple focal hyperplasia at 200 and 1200 ppm. Thyroid focal hypertrophy was increased in males, and thyroid focal hyperplasia in females at 6000

ppm. The incidence of kidney lipofuscin pigmentation was significantly elevated at 200 and/or 1200 and 6000 ppm in both sexes, and was accompanied by in increase in the severity of nephropathy (from moderate to marked in males and from mild to moderate in females).

Statistically significant increases of lesions which had no obvious relationship to compound treatment were found sporadically in the eyes (acute inflammation, 6000 ppm), the adrenal medulla (focal hyperplasia, 75 and 200 ppm), and the sternum bone marrow (granulocytic hyperplasia, 200 ppm) in males and in the retina (atrophy, 200 and 6000 ppm) and clitoral gland (chronic inflammation, 200 ppm) in females. The statistically significantly increased incidence of pituitary focal hyperplasia in 6000 ppm females may have been caused by the treatment-induced increase in TSH production, though it was not accompanied by an increase in organ weight. Leydig cell hyperplasia in the testis was both statistically decreased (6000 ppm) and increased (75, 200, and 1200 ppm), though historic control rates were not provided to establish the biological significance of the changes. The effects observed in the adrenal cortex of males (fat depletion at 1200 and 6000 ppm, focal necrosis at 6000 ppm) and females (lipidosis at 200, 1200, and 6000 ppm) may have been treatment-related, perhaps due to stress and/or changes in lipid metabolism, though they were not significant toxicologically. The chemically induced hyperparathyroidism in males likely caused or contributed to effects including renal failure, eye corneal calcification, heart medial calcification and fibrosis, femur and sternum osteoclastic resorption, and stomach and coagulating gland calcium deposition. Thyroid calcium deposition and lung medial calcification also occurred in females, although no changes were seen in the parathyroids. A significant dose-dependent increase in skin follicular cell atrophy occurred in females, reaching statistical significance at 200 and 6000 ppm. This increase parallels the increases in alopecia in females, both effects possibly being secondary results of lowered thyroid hormones.

The incidence of several lesions was statistically decreased in males (spleen hemosiderin deposition and extramedullary hematopoiesis, liver perilobular fatty degeneration, eosinophilic and basophilic cell foci, femur bone marrow microgranuloma, adrenal cortex focal hyperplasia, thyroid parafollicular cell focal hyperplasia) and in females (liver perilobular fatty degeneration and basophilic cell foci, kidney calcium deposition, and spleen hemosiderin deposition and extramedullary hematopoiesis), though these changes were not biologically relevant. The results for males are shown in Table 13, and for females in Table 14. Information about the severity of the lesions is not included because it was not tabulated for the total main study group by the authors.

TABLE 13. INCIDENCE OF NONNEOPLASTIC LESIONS WHICH WERE STATISTICALLY SIGNIFICANTLY CHANGED IN MALE RATS ADMINISTERED THIOPHANATE METHYL (MAIN STUDY)[a]

Organs/Lesions	Doses (ppm)				
	0	75	200	1200	6000
Lesions having increased incidence in males					
Eye/ Inflammation, acute[b]	2/50	2/22	3/31	2/50	14/55**
/Calcification, corneal	29/50	5/12	15/31	40/50*	28/55
Heart/ Medial calcification[b]	2/50	2/17	1/24	2/50	24/55***
/Fibrosis[b]	6/50	10/17***	14/24***	17/50**	32/55***
Bone marrow of sternum/ Hyperplasia, granulocytic	5/50	3/17	9/24**	2/49	4/54
Femur/ Resorption, osteoclastic[b]	2/50	2/17	2/24	7/50	35/55***
Sternum/ Resorption, osteoclastic[b]	2/50	2/17	2/24	7/49	34/54***
Liver/ Hypertrophy and lipofuscin pigmentation[b]	0/50	0/50	0/50	19/50***	46/55***
/ Focal fatty degeneration[b]	9/50	7/50	10/50	12/50	27/55***
/ Necrosis, focal[b]	0/50	2/50	2/50	2/50	6/55*
/ Multiple focal hyperplasia	2/50	6/50	9/50*	9/50*	5/55
Stomach/ Calcium deposition[b]	1/50	2/19	2/23	5/50	29/55***
Coagulating gland/ Calcium deposition[b]	0/48	0/17	0/24	0/50	7/53**
Thyroid/ Hypertrophy and Hyperplasia[b]	0/50	0/48	0/50	13/50***	53/55***
/Focal hypertrophy[b]	3/50	2/48	2/50	3/50	15/55**
Parathyroid/ Hypertrophy and Hyperplasia[b]	6/48	1/47	2/48	7/47	34/50***
Kidney/Lipofuscin pigmentation[b]	1/50	2/50	6/50*	8/50*	8/55*
Testis/ Leydig cell hyperplasia	13/50	26/48**	29/44***	28/50**	7/55*
Adrenal cortex/ Fat depletion[b]	3/50	0/50	4/49	14/50**	19/55***
/Focal necrosis[b]	0/50	0/50	0/49	0/50	5/55*
Adrenal medulla/ Focal hyperplasia	3/50	13/50**	11/50*	8/50	6/55
Lesions having decreased incidence in males					
Spleen/ Hemosiderin deposition	47/50	13/21**	14/24***	31/50	50/55
/Hematopoiesis, extramedullary	46/50	12/21**	15/24**	35/50**	48/55
Liver/ Perilobular fatty degeneration[b]	19/50	14/50	16/50	5/50***	0/55***
/Eosinophilic cell focus[b]	25/50	27/50	22/50	20/50	8/55***
/Basophilic cell focus[b]	35/50	27/50*	23/50**	24/50*	10/50***
Bone marrow of femur/ Microgranuloma[b]	7/50	0/17	1/24	2/50	0/55**
Adrenal cortex/ Focal hyperplasia[b]	35/50	33/50	29/50	30/50	14/55***
Thyroid/ Parafollicular cell focal hyperplasia[b]	16/50	8/48*	8/50*	11/50	4/55***

[a]Data taken from Tables 31 and 33, MRID No. 428966-01, and is presented as the number of animals showing a lesion/number of animals examined. Statistical significance was calculated by the reviewer using the Fischer exact test. Significantly different from control: * $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$

[b]Cochran-Armitage trend test indicated there was a dose-related response ($p < 0.05$) for the four tested doses.

TABLE 14. INCIDENCE OF NONNEOPLASTIC LESIONS WHICH WERE STATISTICALLY SIGNIFICANTLY CHANGED IN FEMALE RATS ADMINISTERED THIOPHANATE METHYL (MAIN STUDY)[a]

Organs/ Lesions	Doses (ppm)				
	0	75	200	1200	6000
Lesions having increased incidence in females					
Skin/ Follicular atrophy	13/50	7/19	13/21**	17/50	21/50*
Eye/ Retinal atrophy[b]	9/50	2/20	8/16*	4/50	26/50***
Liver/ Hypertrophy and lipofuscin[b]	0/50	0/50	0/50	28/50***	42/50***
Kidney/ Lipofuscin pigmentation[b]	4/50	5/50	6/50	18/50***	44/50***
Thyroid/ Calcium deposition /Hypertrophy and hyperplasia[b] /Hyperplasia, focal[b]	3/50 1/50 0/50	1/49 1/49 1/49	0/50 0/50 0/50	1/50 23/50*** 4/50	35/50*** 49/50*** 6/50*
Lung/ Medial calcification	16/50	15/50	18/50	24/50*	18/50
Pituitary/ Focal hyperplasia[b]	18/50	15/50	13/50	23/49	32/50**
Adrenal cortex/ Lipidosis[b]	5/50	8/50	13/50*	17/50**	14/50*
Clitoral gland/ Chronic inflammation	10/47	3/9	5/8*	3/12	6/48
Lesions having decreased incidence in females					
Liver/Perilobular fatty degeneration[b] /Basophilic cell focus[b]	23/50 39/50	25/50 39/50	22/50 44/50	12/50* 38/50	2/50*** 17/50***
Kidney/Calcium deposition[b]	48/50	46/50	42/50	42/50	27/50*
Spleen/ Hemosiderin deposition /Hematopoiesis, extramedullary	45/50 45/50	7/16*** 6/16***	7/13** 7/17***	8/19*** 9/17**	38/50 37/50

[a]Data taken from Tables 32 and 34, MRID No. 428966-01, and is presented as the number of animals showing a lesion/number of animals examined. Statistical significance was calculated by the reviewer using the Fischer exact test. Significantly different from control: * $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$

[b]Cochran-Armitage trend test indicated there was a dose-related response ($p < 0.05$) for the four tested doses.

2) Neoplastic – At the interim sacrifice, one or two neoplasms were found in several rat tissues (no statistical significance). In males, there was one pituitary adenoma, one thyroid adenoma, and one external auditory canal Zymbal gland carcinoma at 1200 ppm. In females, one subcutaneous fibrosarcoma, one lung fibrosarcoma, and 2 pituitary adenomas were found in control (untreated) females, whereas a uterine endometrial stromal polyp was found at both 0 and 200 ppm.

In the main study, results indicated that the most significant treatment-related induced neoplasm was thyroid follicular cell (FC) adenoma in males. The incidence of adenoma was dose-related, statistical significance being achieved ($p < 0.01$) in the 6000 ppm males. There was also a small, dose-related (statistically non-significant) increase in the incidence of thyroid FC adenocarcinoma in the males. Numerous thyroid pathological changes (hypertrophy and hyperplasia, focal

hypertrophy, increased organ weight, lowered hormone levels) were associated with the neoplastic lesions. In females, there was a statistically non-significant dose-related increase in thyroid FC adenoma. Because the FC adenoma was almost exclusively seen at the two highest doses in both males and females, the adenoma may be a threshold response.

Two other types of tumors were also statistically significantly elevated in the rats, though it was unclear whether they were caused by compound administration: adrenal medullary pheochromocytoma in males (at 75, 200, and 1200 ppm) and spleen mononuclear cell leukemia in both males (at 75 and 200 ppm) and females (at 75, 200, and 1200 ppm). The incidence of adrenal medullary pheochromocytoma had an inverse relationship with dose (being highest at 75 ppm), and was paralleled by an increased incidence of focal hyperplasia at 75 and 200 ppm. The lack of other pathological changes in male rats at 75 and 200 ppm suggests that the pheochromocytoma was not biologically relevant; historical controls were not provided for reference. The increase in spleen mononuclear cell leukemia in both sexes was seen at only intermediate doses where just 13-24 (instead of 50) animals were examined. Significant gross, clinical, or microscopic changes in the spleen did not accompany the neoplasia. The persistence of the increased incidence of leukemia in female spleens and the finding of its metastasis to the liver (statistically significant at 1200 ppm) and mesenteric lymph nodes (statistically significant at 75 and 1200 ppm) suggests the effect may be biologically significant in this sex. Historical controls were not provided for comparison. Several other organs had statistically significant increases in tumors which were likely spontaneous events, as there was no dose-response and the tumors occurred at only one intermediate dose: skin papilloma (at 75 ppm) and pituitary adenoma (at 200 ppm) in males and mammary gland fibroadenoma in females (at 1200 ppm). The neoplastic lesions in males and females are summarized in Table 15.

TABLE 15. TOTAL INCIDENCE OF NEOPLASTIC LESIONS IN RATS ADMINISTERED THIOPHANATE METHYL (UNSCHEDULED DEATH AND TERMINAL SACRIFICE ANIMALS COMBINED)[a]

Organs/Lesions	Doses (ppm)				
	0	75	200	1200	6000
Males					
Skin/Papilloma (benign)	0/49	4/24*	2/31	3/50	2/55
Spleen/Mononuclear cell leukemia (malignant)	4/50	7/21*	9/24**	9/50	6/55
Adrenal medulla/ Pheochromocytoma (benign)	0/50	9/50**	6/50*	5/50*	1/55
Pituitary/Adenoma (benign)	23/50	16/49	31/49*	25/49	2/55
Thyroid/ FC adenoma[b] /FC adenocarcinoma[b] /C-cell adenoma /C-cell adenocarcinoma	1/50 0/50 12/50 2/50	0/48 0/48 10/48 0/48	0/50 0/50 13/50 1/50	3/50 0/50 12/50 0/50	12/55** 3/55 3/55 0/55
TOTAL number of animals with tumors	50/50	49/50	48/50	49/50	46/50
Females					
Mammary gland/Fibroadenoma (benign)	4/50	3/18	3/12	10/21***	5/50
Spleen/Mononuclear cell leukemia (malignant)	4/50	8/16***	6/13**	13/21***	9/50
Mesenteric lymph node /Mononuclear cell leukemia (metastatic)	1/50	3/12*	0/8	4/13**	4/50
Liver/Mononuclear cell leukemia (metastatic)	4/50	7/50	6/50	12/50*	9/50
Thyroid/FC adenoma /FC adenocarcinoma /C-cell adenoma /C-cell adenocarcinoma	0/50 0/50 6/50 0/50	0/49 0/49 9/49 1/49	0/50 0/50 8/50 1/50	1/50 0/50 9/50 0/50	2/50 0/50 5/50 0/50
TOTAL number of animals with tumors	39/50	36/50	33/50	40/50	33/50

[a]Data taken from Tables 31-38 (pages 337-443), MRID No. 428966-01, and is presented as the number of animals showing a lesion/number of animals examined. A description of the tumor type is enclosed in the parentheses. Statistical significance was calculated by the reviewer using the Fischer exact test. Significantly increased relative to control: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

[b]Cochran-Armitage trend test indicated there was a dose-related response ($p < 0.05$) for the four tested doses.

D. DISCUSSION

This study was well conducted. However, there were two omitted items that made interpretation of the study difficult. These were lack of historical control data for certain neoplastic lesions and the lack of combined incidences of neoplastic lesions for unscheduled deaths and terminal sacrifice. These omissions are discussed later. **The study is graded Core Guideline and meets the regulatory requirement for a chronic feeding/oncogenicity study in rats (83-5) for thiophanate methyl.** The following paragraphs summarize and discuss in more detail the effects observed in the study.

Male rats fed 1200 and 6000 ppm had significantly lowered mean body weights (73-84% of controls) and net body weight gains (63-79% of controls) at study termination. Significant weight effects were also seen in 6000 ppm females, the mean body weight and net weight gain being 78% and 69% of controls, respectively, after 104 weeks. Food efficiency was depressed in both sexes at 1200 and 6000 ppm, though total food consumption was unchanged, indicating compound toxicity was causing the decreased relative weight gain. Significant effects on mortality were only seen in the 6000 ppm group males, just 2/55 rats survived to the end of the 2-year study. Ophthalmoscopic examination revealed no treatment-related findings in any of the dose groups.

Statistically significant and dose-related changes were found in hematology parameters in both sexes at 1200 and 6000 ppm. The RBC (males only), hematocrit, hemoglobin, MCV, MCH, and MCHC were decreased, whereas the platelet count and/or WBC were increased at one or more test times. The changes seen were relatively minor, and not biologically significant. In males, the mild anemia correlated with the clinically observed pale discoloration of the skin. It is not obvious how compound treatment caused these effects; there was no evidence of decreased hematopoiesis in the bone marrow or spleen.

The liver, kidneys, and thyroid were the major target organs for thiophanate methyl toxicity, while minor effects were also observed in the adrenal cortex of both sexes of rats. Liver weights (absolute and relative) were significantly increased in both 1200 and 6000 ppm males and females, and there was some dark discoloration (not statistically significant). Microscopic examination revealed centrilobular hepatocellular hypertrophy and lipofuscin pigmentation in male and female 1200 and 6000 ppm rats at both the interim and final sacrifice. The total serum cholesterol of 1200 and/or 6000 ppm male and female rats was elevated throughout the 2-year experiment. The study authors noted that hepatocellular hypertrophy was correlated with the induction of microsomal enzymes, total cholesterol, and liver cell proliferation in several additional short-term experiments (see Appendix pages A-1 and A-2 of this report). The induction of hepatic enzymes may account for some of the increased total serum protein seen in the 2-year study in both sexes at several test times.

The absolute and relative kidney weights were increased and kidney color or surface texture were altered in both sexes of 1200 and 6000 ppm animals. Alterations in clinical blood chemistry parameters indicative of renal damage in 1200 and 6000 ppm rats included increased BUN and serum creatinine, increased urine protein and ketone bodies, and decreased serum albumin. Nephropathy was exacerbated in male and female 1200 and/or 6000 ppm rats, and was generally accompanied by lipofuscin pigmentation. The nephropathy was dose-related, increased in severity with time, and was more severe in males than in females. Renal failure was the most common cause of death among 6000 ppm group male rats.

In the adrenal cortex, the incidence of lipidosis was significantly increased in the 6000 ppm interim sacrifice male rats, and in the interim sacrifice (1200, 6000 ppm) and main study (200, 1200, 6000 ppm) female rats. (The incidence of fat depletion and focal necrosis were also significantly elevated in the main study 1200 and/or 6000 ppm males). The authors suggest that the lipidosis may be due to a disturbance of lipid metabolism (which is also manifest as increased serum cholesterol) or to a general stress response (increased endogenous steroid production). The biological significance of the adrenal cortical changes was relatively minor.

A NOEL of 200 ppm (8.8 mg/kg/day for males and 10.2 mg/kg/day for females) was identified for male and female rats fed thiophanate methyl in the diet for 2 years, based upon the lack of significant treatment-related toxic effects at this dose, while the next higher dose (1200 ppm) was identified as the LOEL (54.4 mg/kg/day for males and 63.5 mg/kg/day for females).

A significant ($p<0.01$), dose-dependent increase in thyroid follicular cell adenoma was seen in 6000 ppm group males, as well as a non-significant increase in FC adenocarcinoma. A small non-statistically significant increase in FC adenoma occurred in 6000 ppm females. The thyroid neoplasia was correlated with numerous clinical, macroscopic, and microscopic changes in both sexes at 1200 and/or 6000 ppm. Thyroid weights (absolute and relative) were significantly increased, there was a marked decrease in T_3 and/or T_4 levels and an increase in TSH levels (with no significant gross or microscopic changes in the pituitary), and diffuse hyperplasia and hypertrophy and focal hyperplasia occurred in follicular cells, which are responsible for T_3 and T_4 production. Because the thyroid adenoma and toxic effects were seen almost exclusively at 1200 and 6000 ppm, these lesions appear to be a threshold response to the thyroid-pituitary hormonal imbalance, the chronic overstimulation of thyroid FC cells culminating in neoplasia. The study authors conducted a series of short-term experiments (summarized on Appendix pages A-1 and A-2) using thiophanate methyl, propylthiouracil (PTU), and phenobarbital (PB) to study the mechanism by which thiophanate methyl causes thyroid toxicity. Their found that thiophanate methyl behaved in some ways like PTU, a known thyroid peroxidase inhibitor (caused increased thyroid weight, depressed T_3 and T_4, increased TSH, inhibited porcine thyroid peroxidase activity

in vitro), while also inducing hepatic microsomal enzymes, as did phenobarbital. They also showed that thyroid hypertrophy was reversed upon removal of thiophanate methyl, and that simultaneous addition of thiophanate methyl and T_4 negated effects caused by the compound alone (and had no effects on liver hypertrophy and total serum cholesterol). Judging by these data, it cannot be determined whether the thiophanate methyl-induced thyroid toxicity was due to inhibition of thyroid peroxidase (and hormone synthesis) or the increased elimination of T_4 by hepatic drug metabolism enzymes, or a combination of the two.

Only a few tumors were found in interim sacrifice animals, whereas in the main study group incidences of a variety of neoplasms were statistically significantly elevated. The data indicated that some of the tumors were incidental to treatment (skin papilloma and pituitary adenoma in males and mammary gland fibroadenoma in females), while the biological significance and relationship to treatment of two tumor types was equivocal (spleen mononuclear cell leukemia in males and females and adrenal medullary pheochromocytoma in males). The MTD appears to have been achieved in the study for both males (1200 ppm or 54.4 mg/kg/day) and females (6000 ppm or 334.7 mg/kg/day). The MTD was exceeded at the dose showing a statistically significant increase in thyroid FC adenoma in males (6000 ppm), as only 2/55 rats survived to study termination.

E. STUDY DEFICIENCIES

There were no deficiencies of sufficient gravity to invalidate the interpretation of the results of this 2-year study. There were, however, several notable shortcomings, a major one being the lack of historical control data to aid in determining the significance of results. There were several instances where the reviewer would have been more comfortable with the conclusions drawn from the obtained results (e.g. lack of significance for male adrenal pheochromocytoma and male and female spleen mononuclear cell leukemia) if there was supporting historical control data.

The survival of male rats in the 6000 ppm dose group was below the guideline requirement for a 2-year chronic feeding/oncogenicity study in rats, though the three lower doses tested were acceptable to fulfill the requirement. The mortality data would have better reflected treatment-related effects in the 6000 ppm male group if the 8 males which died due to treatment-unrelated injury during weeks 11 and 12 had been excluded.

The macroscopic and microscopic animal data for terminal sacrifice and unscheduled-death rats should have been combined in tabular form because these animals were all treated and assessed for toxicity in the same manner, and together constituted the main study group. The interim-sacrifice tumor data should not have been combined with the main study data to tabulate the total incidence of neoplasms because the animals in the interim-sacrifice group were not allowed

the same amount of time to develop neoplasms (and most of the neoplasms found in this study did not develop until the second year).

The investigators also did not provide adequate quantitative data from their range-finding studies.

APPENDIX

Summary of the Mechanistic Investigation of the Effect of Thiophanate Methyl on Thyroid and Liver

A series of 6 acute short-term experiments were carried out by the same investigators to explore the mechanism by which thiophanate methyl causes pathological changes in the thyroid and liver. The experiments are detailed in Annex 5 (pages 529-535) of the study (MRID No. 428966-01). Male and female F344 rats (5/group), male ICR mice (5/group), and porcine thyroid microsomes were treated with either thiophanate methyl (TM), propylthiouracil (PTU, a thyroid hormone synthesis inhibitor), or phenobarbital (PB, a liver microsomal enzyme inducer). Exposure was for 2 and/or 8 days in food (TM, 6000 ppm; PB, 500 ppm) or distilled water (PTU, 1000 ppm). The Student's t-test and Mann-Whitney U-test were used to assess the significance between control and dosed groups (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). The individual experiments and their results are summarized as follows:

Experiment 1 – Rats were treated with TM, PB, or PTU for 2 or 8 days and killed. The effects on liver and thyroid weights and serum total cholesterol were measured, and radioimmunoassay was used to quantitate levels of thyroid hormones (T_3 and T_4), and TSH. The results are presented in Annex 5, Table 1 (page 532, top), MRID No. 428966-01. After 2 and/or 8 days, TM and PTU significantly decreased T3 and T4 levels and increased TSH levels, thyroid weight, and total serum cholesterol. PB caused either less severe or no effect on these parameters. PB and TM both caused liver hypertrophy, while there was a marginal weight decrease in the PTU treated group after 2 days.

Experiment 2 – Female rats were treated for 8 days with TM or PB, and thyroid weight was measured after sacrifice either at day 8 or at day 16. Results show that thyroid weights returned to normal 8 days after TM treatment was discontinued, after having more than doubled during the 8-day treatment (see Table 2, page 532, MRID No. 428966-01). PB caused no change in thyroid weight compared to controls after 8 or 16 days.

Experiment 3 – Rats were treated with TM, T_4 (subcutaneous daily injection of 30 μg/kg), or a combination of the two for 8 days to see whether supplementing with exogenous T_4 would change the effects caused by TM treatment alone. The results (presented in Annex 5, Table 3 (page 533) of MRID No. 428966-01) show that T_4 supplementation suppressed thyroid hypertrophy and TSH increases caused by TM, though it had no effect on the induced liver hypertrophy or increased total cholesterol.

Experiment 4 – Microsomes were isolated from the livers of Experiment 1 rats treated with TM or PB and sampled on day 8. The microsomal protein, P-450, cytochrome b5, NADPH-cytochrome c reductase (NCCR), and UDP-glucuronosyltransferase (UDP-GT) activities were measured. UDP-GT is believed to affect T4 excretion by the liver. The results (Table 4 of MRID No. 428966-01, p. 533) indicate that both

TM and PB induced production of almost all these drug-metabolizing enzymes and protein (NCCR levels in TM-treated animals were unchanged).

Experiment 5 – The microsome fraction was isolated from commercially obtained porcine thyroids and peroxidase activity was measured (guaiacol assay method) in the presence of TM (10^{-3} to 10^{-4} M) or PTU (10^{-4} to 10^{-6} M). TM and PTU both inhibited thyroid peroxidase activity, though the inhibition by PTU was 30-fold greater. (Results are presented in Table 5 (page 533, bottom) of MRID No. 428966-01.)

Experiment 6 – The ability of TM and PB to cause proliferation of liver cells was assayed by staining cells of treated male F344 rats and ICR mice for proliferating cell nuclear antigen (PCNA). Animals were administered the compounds for 2 or 8 days, sacrificed, and liver paraffin sections were prepared for staining. Microscopic examination revealed that there were significantly more PCNA positive cells on day 2 in both in mice and in rats, and on day 8 in mice. The results are summarized in Tables 6-1 and 6-2 (p. 534) of MRID No. 428966-01.

Summary/Conclusions

Treatment of rats for 2 or 8 days with 6000 ppm thiophanate methyl caused thyroid and liver hypertrophy, decreased thyroid hormone levels (T_3 and T_4), elevated TSH, and induced liver microsomal enzymes. Treatment caused proliferation of liver cells in mice and rats, and an *in vitro* study showed thyroid peroxidase was inhibited. Thyroid hyperplasia caused by TM treatment was reversible.

PB and PTU both caused some of the same effects on the liver and thyroid as thiophanate methyl. PTU, a known thyroid hormone synthesis inhibitor, decreased thyroid hormone levels, increased TSH levels, caused thyroid hypertrophy, and inhibited porcine thyroid peroxidase *in vitro*. Like thiophanate methyl, PB caused liver hypertrophy, liver cell proliferation, and induced liver microsomal drug-metabolizing enzymes (including UDP-GT, which helps clear T_4 from the liver), though it caused only minimal increase in TSH and did not cause thyroid hypertrophy. Thus, the mechanism of action of TM appears to be more like that of PTU than like PB, i.e., inhibition of thyroid hormone metabolism. Consistent with this, and suggesting the involvement of a negative feedback loop, adding exogenous T_4 together with TM negated the effects of TM on thyroid hypertrophy and hormone levels and on TSH levels, though it had no effect on liver weight or total cholesterol.

Results

Exp 1. TM, as well as PTU, caused decreases in T_4 and T_3 levels and an increase in TSH level on days 2 and 8. But, the TM group showed rapid recovery compared with the PTU group. In the PB group, only a slight increase of TSH level was noted.

Table 1. Liver and thyroid weights, T3, T4 and TSH levels

	Day	Control 0 ppm	TM 6000 ppm	PTU 1000 ppm	PB 500 ppm
DI (mg/kg/day)	2	0	502	42	55
	8	0	519	74	49
Liver wt (g)	2	6.4±1.0	8.2±1.0*	4.9±0.6*	ND
	8	7.6±1.2	11.1±1.6**	7.3±0.9	10.4±1.4**
Thyroid wt (mg)	2	18±4	19±2	21±1	ND
	8	23±4	53±7***	65±2***	25±4
T_3 (ng/dl)	2	93±9	56±10**	38±5***a	ND
	8	95±3	80±3***	24±2***	107±6**
T_4 (μg/dl)	2	5.5±.5	3.4±.7**	4.8±.9a	ND
	8	5.6±.6	5.0±.7	1.9±.3***	6.2±.4
TSH (ng/100μl)	2	0.47±.14	1.10±.53	2.01±0.67**a	ND
	8	0.48±.07	2.37±.83***	5.43±1.29***	0.63±.06**
T.Cho (mg/dl)	2	69.8±6.3	92.7±7.7	69.6±10.8	ND
	8	58.3±3.3	89.7±10.9***	89.5±7.7 ***	71.8±4.0**

DI: Drug intake, a: 4 rats were measured.

Exp 2. TM caused hypertrophy of the thyroid by day 8. But, it returned to normal after an 8-day recovery period (Day 16). No significant change was noted in the thyroid weights of the PB group (Table 2).

Tale 2. Recovery of thyroid weights

	Day	Dose	Control 0 ppm	TM 6000 ppm	PB 500 ppm
Thyroid weight (mg)	8		15±5	36±8**	17±2
	16		21±5	25±4	20±2
Body weight (g)	8		127.9±6.8	129.4±5.8	135.3±6.3
	16		158.4±8.0	162.7±12.1	165.4±6.6

Exp 3. TM caused hypertrophy of the thyroid and liver (Table 3). The hypertrophy of the thyroid was completely suppressed by concomitant subcutaneous injection of T_4. The increases of liver weights and cholesterol values were not affected by the T_4 supplementation.

Table 3. Body weights, Thyroid weights, Liver weights and Cholesterol values

	Control	T4	TM	TM+T4
Body weight (g)	216.6 ±7.6	208.5 ±8.5	210.7 ±8.1	207.3 ±8.8
Thyroid wt (mg)	19.2 ±4.7	17.6 ±2.5	43.8 ±4.7***	19.4 ±4.9
Liver wt (g)	8.09±.39	7.56±.53	11.16±.70***	10.73±.74***
TSH (ng/100μl)	0.50±.09	0.33±.02**	2.32±.93**	0.51±.16
T.Cho. (mg/dl)	50.8 ±3.5	47.0 ±3.8	67.8 ±6.1**	69.1 ±5.2***

Exp 4. Enzyme induction. TM as well as PB induced microsomal P-450, cytochrome b5 (C b5), protein and UDP-glucuronosyltransferase (UDP-GT) (Tales 4). PB induced NADPH-cytochrome c reductase (NCCR), also.

Table 4. Drug metabolizing enzymes and protein (Mean and SD)

Dose	Control 0 ppm	TM 6000 ppm	PB 500 ppm
P-450[1]	0.62±0.05	1.01±0.05***	1.16±0.09***
C b5[1]	0.44±0.03	0.72±0.05***	0.60±0.02***
NCCR[2]	433 ±45	453 ±94	590 ±47**
Protein[3]	21.3±0.9	25.1±1.7**	26.9±2.2**
UDP-GT[2]	20.6±3.9	69.2±19.5**	43.9±6.7**

N=4, [1]:nmol/mg, [2]:nmol/min/mg microsomal protein, [3]:mg/g liver.

Exp 5. Effects on porcine thyroid peroxidase. TM inhibited thyroid microsomal peroxidase in swine (Table 5). The ED50 of inhibition with TM was 30-fold smaller than that of PTU.

Table 5. Inhibition of porcine thyroid peroxidase

	TM	PTU
ED50	$6x10^{-4}$ M	$2x10^{-5}$ M
EDO	$8x10^{-5}$ M	$4x10^{-7}$ M

Exp 6. Proliferation of liver cells. In mice, PCNA positive cells were increased on days 2 and 8. In rats, they were increased on day 2, but not on day 8 (Table 6). TM seemed to maintain cell proliferation of hepatocytes in mice than in rats, when administered TM or PB for 8 days.

Table 6-1. Number of PCNA positive cells in the liver of mice

	Day	Mice[1] Control	TM (6000 ppm)	PB (500 ppm)
PCNA	2	3±3	27±11**	175±111**
	8	0±0	21±13**	22±11**
Liver wt (g)	2	2.05±.18	2.49±.28*	2.49±.17**
	8	2.10±.10	2.67±.20**	2.85±.27***

1:0.28 mm^2x20 fields (total 5.6 mm^2), N=5/group.

Table 6-2. Number of PCNA positive cells in the liver of rats

	Day	Rats[2] Control	TM (6000 ppm)	PB (500 ppm)
PCNA	2	19±6	113±24**	61±21**
	8	20±6	12± 6	17± 6
Liver wt (g)	2	9.74±.25	11.68±.52***	11.27±.40***
	8	10.11±.62	13.19±.60***	12.67±.48***

2:0.05 mm^2x20 fields (total 1 mm^2). N=5/group.

DISCUSSION

As shown in Table 1, TM caused decreases of serum T_4 and T_3 levels and an increases of TSH level and the thyroid weight on days 2 and/or 8. There were close correlation between TSH level and thyroid weight. The T_4 supplementation to rats treated with TM counteract the hypertrophy and TSH response (Table 3). These data indicate that TM causes the hypertrophy of thyroid by negative feed back mechanism. The effect of TM on thyroid was reversible as shown in Table 2.

PTU is a well known inhibitor of thyroid hormone synthesis. The effects of PTU on thyroid weights and hormones levels were similar to those of TM. Moreover, TM inhibited thyroid peroxidase as PTU did (Table 5). However, the inhibition was 30 folds weaker in TM than PTU.